IN THE CLAIMS:

Please amend claims 1, 3-6, 16, 45, 51, 52, 53, 57, 59 and 63 as follows. This listing of claims replaces all prior versions, and listings of claims, in the application.

LISTING OF CLAIMS:

1. (Currently Amended) A method of producing and identifying a mammalian protease mutein that inactivates an activity of a target protein, wherein:

the target protein is a protein that is involved with a disease or pathology in a mammal, whereby inactivating an activity of the target protein can treat the disease or pathology; and

the method ~~comprising~~ comprises the steps of:

(a) producing a library comprising protease muteins comprising mutation(s) of a serine protease scaffold and/or biologically active portions thereof at an amino acid position selected from among amino acid residues ~~between 58 and 64,~~ 97, 98, 99, 100, 171, 174, 180, 189, 190, 191, 192, 215, 217, 218 , ~~and~~ 226 and amino acid residues between amino acid 58 and amino acid 64, inclusive, whereby numbering of amino acid residues is by chymotrypsin numbering;~~, wherein:~~

~~each different mutein protease in the library is a member of the library;~~

~~each member of the library has N mutations relative to a wild-type mammalian protease scaffold or a biologically active portion thereof; and~~

~~N is a positive integer;~~

(b) contacting members of the library with a target protein or with a polypeptide comprising a substrate sequence that is present in the target protein, wherein:

the target protein is ~~selected from among~~ a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor ~~and~~ or a signaling protein that regulates apoptosis; and

cleavage of a substrate sequence in the target protein inactivates an activity of the target protein; and

(c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the target protein or the polypeptide comprising the substrate sequence that is present in the target protein;

(d) ~~based on the measured activity and/or specificity,~~ identifying protease mutein(s) ~~members of~~ from the library that have an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence or the target protein, relative to the wild-type

mammalian protease scaffold based on the measured activity and/or specificity in step (c); and

(e) testing the identified protease mutein(s) or biologically active portion thereof for cleavage and inactivation of an activity of the target protein that contains the substrate sequence to verify that the function of the target protein has been inactivated by the cleavage event, wherein:

the protease mutein or biologically active portion thereof cleaves a substrate sequence in the target protein that is different from a native substrate sequence of the wild-type mammalian protease scaffold; and

cleavage of the substrate sequence in the target protein inactivates an activity of the target protein, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology.

2. (Cancelled)

3. (Currently Amended) The method of claim 1, wherein ~~N is an integer~~ the number of mutation(s) of a serine protease scaffold and/or biologically active portions thereof is between 1 and 20.

4. (Currently Amended) The method of claim 3, wherein ~~N is an integer~~ the number of mutation(s) of a serine protease scaffold and/or biologically active portions thereof is from 1-5.

5. (Currently amended) The method of claim 3, wherein ~~N is an integer~~ the number of mutation(s) of a serine protease scaffold and/or biologically active portions thereof is from 5-10.

6. (Currently amended) The method of claim 3, wherein ~~N is an integer~~ the number of mutation(s) of a serine protease scaffold and/or biologically active portions thereof is from 10-20.

7. (Previously presented) The method of claim 1, wherein the wild-type mammalian protease is selected from among trypsin, chymotrypsin, subtilisin, MTSP-1, granzyme A, granzyme B, and granzyme M, elastase, chymase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, cruzain, and urokinase plasminogen activator (uPA).

8. (Cancelled)

9. (Previously presented) The method of claim 1, wherein the pathology is selected from among rheumatoid arthritis, sepsis, cancer, acquired immunodeficiency syndrome, respiratory tract infections, influenza, cardiovascular disease and asthma.

10. (Canceled)

11. (Original) The method of claim 1, wherein the target protein is involved in apoptosis.

12. (Previously presented) The method of claim 11, wherein the target protein is caspase-3, VEGF or VEGF-R.

13. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 10-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

14. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 100-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

15. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 1000-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

16. (Currently amended) The method of claim 1, further comprising the steps of:

~~(e)~~ (f) identifying the mutation(s) contained in a first mutein protease and a second mutein protease identified in step ~~e)~~ d) as having increased cleavage activity and/or altered specificity;

~~(f)~~ (g) producing a third mutein protease containing the mutations of the first mutein protease and the mutations of the second mutein protease; and

~~(g)~~ (h) measuring the cleavage activity and/or substrate specificity of the third mutein protease to determine its cleavage activity and/or specificity for the substrate sequence compared to the first mutein protease or second mutein protease.

17-44. (Canceled)

45. (Currently amended) The method of claim 1, further comprising:

repeating steps ~~a) d)~~ a)-e) iteratively to produce a further library of protease muteins from the identified muteins each with increased cleavage activity and/or altered substrate specificity; and

identifying among the further library, a mutein protease having altered specificity and/or increased cleavage activity relative to the wild-type mammalian protease.

46. (Cancelled)

47. (Cancelled)

48. (Previously presented) The method of claim 1, wherein the substrate sequence is a sequence in a target protein that is a human protein.

49.-50. (Cancelled)

51. (Currently amended) The method of claim [[50]] 1, wherein the member of the library identified in step (d) has the highest measured cleavage activity for the substrate sequence among the identified members of the library.

52. (Currently amended) The method of claim 1, ~~further comprising~~ wherein testing the identified protease in step (e) comprises ~~the steps of~~ providing at least one mutein protease identified in step (d), and testing the mutein protease in an *in vivo* assay for inactivation of an activity of the target protein.

53. (Currently Amended) A method of producing and identifying a mammalian protease mutein that inactivates an activity of a target protein, wherein:

the target protein is a protein that is involved with a disease or pathology in a mammal, whereby inactivating an activity of the target protein can treat the disease or pathology; and

the method ~~comprising~~ comprises the steps of:

(a) producing a library comprising protease muteins comprising mutation(s) of a mammalian protease scaffold and/or biologically active portion thereof at an amino acid position selected from among amino acid residues ~~between 58 and 64,~~ 97, 98, 99, 100, 171, 174, 180, 189, 190, 191, 192, 215, 217, 218, ~~and~~ 226 and amino acid residues between amino acid 58 and amino acid 64, inclusive, whereby numbering of amino acid residues is by chymotrypsin numbering, wherein:

the mammalian protease scaffold is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, cruzain, and urokinase plasminogen activator (uPA);

~~each different protease mutein in the library being a member of the library;~~

~~each member of the library has N mutations relative to a wild-type mammalian protease scaffold or a biologically active portion thereof; and~~

~~N is a positive integer;~~

(b) contacting members of the library with a target protein or with a polypeptide comprising a substrate sequence that is present in the target protein, wherein[[:]]

~~the target protein is involved with a disease or pathology in a mammal; and~~

cleavage of a substrate sequence in the target protein inactivates an activity of the target protein;

(c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the target protein or the polypeptide comprising a substrate sequence that is present in the target protein;

(d) ~~based on the measured activity and/or specificity,~~ identifying protease mutein(s) from ~~those members of~~ the library that have an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence or the target protein relative to the wild-type mammalian protease scaffold based on the measured activity and/or specificity in step (c);

(e) testing the identified mutein protease mutein(s) or biologically active portion thereof for cleavage and inactivation of an activity of the target protein that contains the substrate sequence to verify that the function of the target protein has been inactivated by the cleavage event, wherein:

the protease mutein or biologically active portion thereof cleaves a substrate sequence in the target protein that is different from a native substrate sequence of the wild-type mammalian protease scaffold; and

cleavage of the substrate sequence in the target protein inactivates an activity of the target protein, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with or causes the disease or pathology;

(f) identifying the mutation(s) contained in a first mutein protease and a second mutein protease identified in ~~step (d)~~ step (e) as having increased cleavage activity and/or altered specificity for cleaving the target protein or substrate sequence and that can inactivate an activity of the target protein;

(g) generating a third mutein protease containing the mutations of the first mutein protease and the mutations of the second mutein; and

(h) measuring the cleavage activity and/or substrate specificity of the third mutein protease to determine whether the third mutein protease has increased cleavage activity and/or altered specificity toward the target protein or substrate sequence compared to the first mutein protease or second mutein protease; and

(i) testing the third mutein for cleavage and inactivation of an activity of the target protein that contains the substrate sequence, thereby identifying a protease mutein or a

biologically active portion thereof that inactivates an activity of a target protein that is involved with or causes the disease or pathology.

54. (Cancelled)

55. (Cancelled)

56. (Cancelled)

57. (Currently amended) The method of claim 53, further comprising:

repeating steps ~~a) d)~~ a)-i) iteratively to produce a further library of protease muteins from the identified muteins each with increased cleavage activity and/or altered substrate specificity; and

identifying among the further library, a mutein protease having altered specificity and/or increased cleavage activity for the substrate sequence relative to the wild-type mammalian protease.

58. (Previously presented) The method of claim 53, wherein the substrate sequence is a sequence in a target protein that is a human protein.

59. (Currently amended) A method of producing and identifying a human protease mutein that inactivates an activity of a target protein, wherein:

the target protein is a protein that is involved with a disease or pathology in a human, whereby inactivating an activity of the target protein can treat the disease or pathology; and

the method ~~comprising~~ comprises the steps of:

(a) producing a library comprising protease muteins comprising mutation(s) of a human protease scaffold and/or biologically active portions thereof at an amino acid position selected from among amino acid residues between ~~58 and 64,~~ 97, 98, 99, 100, 171, 174, 180, 189, 190, 191, 192, 215, 217, 218, ~~and~~ 226 and amino acid residues between amino acid 58 and amino acid 64, inclusive, whereby numbering of amino acid residues is by chymotrypsin numbering, wherein:

~~each different protease mutein in the library is a member of the library;~~

~~each member having N mutations relative to a wild-type human protease scaffold or a biologically active portion thereof, wherein N is a positive integer; and~~

the human protease scaffold is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, cruzain, and urokinase plasminogen activator (uPA);

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein, wherein:

the target protein is ~~selected from among~~ a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor ~~and~~ or a signaling protein that regulates apoptosis; and

cleavage of a substrate sequence in the target protein inactivates an activity of the target protein;

(c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the polypeptide comprising the substrate sequence that is present in the target protein or the target protein; and

(d) ~~based on the measured activity and/or specificity,~~ identifying protease mutein(s) from ~~those members of~~ the library that have an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence or the target protein relative to the wild-type human protease scaffold based on the measured activity and/or specificity in step (c); and

(e) testing the identified protease mutein(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence to verify that the function of the target protein has been inactivated by the cleavage event, wherein:

the protease mutein or biologically active portion thereof cleaves a substrate sequence in the target protein that is different from a native substrate sequence of the wild-type human protease scaffold; and

cleavage of the substrate sequence in the target protein inactivates an activity of the target protein, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with or causes the disease or pathology.

60. (Cancelled)

61. (Previously presented) The method of claim 59, wherein the wild-type protease scaffold is Granzyme B or MTSP-1.

62. (Previously presented) The method of claim 59, wherein the target protein is selected from among caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor,

CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2) and cyclin dependent kinase-4 (cdk-4).

63. (Currently amended) A method of producing and identifying a human protease mutein that inactivates an activity of a target protein, wherein:

the target protein is a protein that is involved with a disease or pathology in a mammal, whereby inactivating an activity of the target protein can treat the disease or pathology; and

the method comprises ~~comprising~~ the steps of:

(a) producing a library comprising human protease muteins comprising mutation(s) of a human protease scaffold and/or biologically active portions thereof at an amino acid position selected from among amino acid residues ~~between 58 and 64,~~ 97, 98, 99, 100, 171, 174, 180, 189, 190, 191, 192, 215, 217, 218 , ~~and~~ 226 and amino acid residues between amino acid 58 and amino acid 64, inclusive, whereby numbering of amino acid residues is by chymotrypsin numbering, wherein:

~~each different protease mutein in the library is a member of the library;~~

~~each member having N mutations relative to a wild-type human protease scaffold or a biologically active portion thereof, wherein N is a positive integer; and~~

the human protease scaffold is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, MTSP-1, elastase, chymase, tryptase, chymotrypsin, collagenase, factor Xa, Protein C, plasma kallikrein, plasmin, trypsin, thrombin, complement factor serine proteases, papain, ADAMTS13, endopeptidase, furin, cruzain and urokinase plasminogen activator (uPA); and

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein, wherein:

the target protein is ~~selected from among~~ a caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41,

hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2), ~~and~~ or cyclin dependent kinase-4 (cdk-4); and

cleavage of a substrate sequence in the target protein inactivates an activity of the target protein;

(c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the polypeptide comprising the substrate sequence that is present in the target protein or the target protein; and

(d) identifying protease(s) ~~based on the measured activity and/or specificity, identifying those~~ members of the library that have an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence or the target protein relative to the wild-type human protease scaffold based on the measured activity and/or specificity in step (c); and

(e) testing the identified protease muteins(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence to verify that the function of the target protein has been inactivated by the cleavage event, wherein:

the protease mutein or biologically active portion thereof cleaves a substrate sequence in the target protein that is different from a native substrate sequence of the wild-type human protease scaffold; and

cleavage of the substrate sequence in the target protein inactivates an activity of the target protein, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology.

64. (Cancelled)

65. (Previously presented) The method of claim 63, wherein the human protease scaffold is selected from among granzyme A, granzyme B, granzyme M and MTSP-1.

66. (Previously presented) The method of claim 63, wherein the target protein is selected from among caspase 3, vascular endothelial growth factor and VEGF receptor.

67. (Previously presented) The method of claim 1, wherein the substrate sequence is pre-selected so that its cleavage in the target protein inactivates the target protein.

68. (Previously presented) The method of claim 1, wherein the substrate sequence comprises a P4-P1 tetrapeptide substrate.

69. (Previously presented) The method of claim 1, wherein the target protein or substrate sequence is fluorogenically labeled.

70. (Previously presented) The method of claim 53, wherein the substrate sequence is pre-selected so that its cleavage in the target protein inactivates the target protein.

71. (Previously presented) The method of claim 53, wherein the substrate sequence comprises a P4-P1 tetrapeptide substrate.

72. (Previously presented) The method of claim 53, wherein the target protein or substrate sequence is fluorogenically labeled.

73. (Previously presented) The method of claim 59, wherein the substrate sequence is pre-selected so that its cleavage in the target protein inactivates the target protein.

74. (Previously presented) The method of claim 59, wherein the substrate sequence comprises a P4-P1 tetrapeptide substrate.

75. (Previously presented) The method of claim 59, wherein the target protein or substrate sequence is fluorogenically labeled.

76. (Previously presented) The method of claim 63, wherein the substrate sequence is pre-selected so that its cleavage in the target protein inactivates the target protein.

77. (Previously presented) The method of claim 63, wherein the substrate sequence comprises a P4-P1 tetrapeptide substrate.

78. (Previously presented) The method of claim 63, wherein the target protein or substrate sequence is fluorogenically labeled.